Sterilization of honey for bee feeding

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FROM MANY POINTS of view, honey appears to be an ideal material for the various types of bee feeding. A disadvantage, of course, is the higher cost of honey over sugar, which is widely used. Off-grade, dark, strong-flavored, unmarketable honey would be economically more suited for the purpose. Unfortunately, such use of honey is often not recommended because of the possibility of spreading the serious bee disease, American foulbrood.

Several years ago, at the request of Production and Marketing officials of this Department, we undertook a study of this problem. The objective was the development of a practical method for the sterilization of honey with respect to the organism causing American foulbrood so that the then considerable amounts of low-grade honey in the hands of the Department might be used for bee feeding without possible spread of disease. J. I. Hambleton, of the Section of Bee Culture and Biological Control, urged that sulfa not be relied on for the purpose and also that the treatment be such that the spores of the organism be killed and not simply be rendered nonvirulent.

There was some previous experience to go on. White in 1920 (1) stated that a variation had been observed in the thermal resistance of spores of **Bacillus larvae** obtained from different geographical sources. He found that the most resistant spores studied, when suspended in water, were destroyed by heating at 212° F. for 11 minutes but withstood longer heating when suspended in honey or diluted honey. White found that spores suspended in honey diluted with an equal volume of water could be destroyed by heat at 212° F. but that it might require half an hour or more.

Many beekeepers for a considerable number of years have more or less followed the following instructions (2): "No attempt should be made to recover honey from diseased colonies unless there is a distinct economic saving. Since it is often impossible to ascertain the source of honey purchased on the open market, such honey should not be fed to colonies of bees if it can be avoided. If such honey has to be used, it should first be diluted with an equal volume of water and boiled for an hour in a closed vessel."

Burnside recommended in 1945 (3) that "boiling for 30 minutes can be depended upon to destroy the virulence of spores of **Bacillus larvae** under any ordinary conditions." This was based on a thermal resistance study of spores of **B. larvae** in water, diluted honey and beeswax. Sixty-two colonies of bees received honey containing spores that had been boiled for various times from five minutes to five hours. None showed any signs of disease during the season. All spores that had been boiled in honey showed vegetative growth in the laboratory; all, however, also showed a delayed germination time. Burnside (3) interpreted the loss of virulence as brought about by the increase in germination time of the heated spores beyond the 2¼ day period in which larvae are susceptible to the disease, according to Woodrow (4).

With these requirements in mind, we have worked out a process by which honey can be sterilized with respect to AFB spores. In this work we used honey infected with **B. larvae** spores at a level recommended by other workers in the field, about 150 million per ml.

Approaching the Problem

OUR FIRST APPROACH to the problem was to try to find some chemical agent or material that we could add to the honey that would kill the spores, either alone or in combination with some heating. After many trials, we found several substances that looked promising at a level of 0.1%[3], combined with heating. Sam-

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[3] These are listed on page 13 of reference 5

ples were made up and tested by feeding to honey bees. The chemicals were fair sporicides, but they also killed the bees.

After this experience, we did some fundamental research on the AFB spores. We decided to rely on heat alone and carried out what is called a thermal-death-time study on the organism. This means that we determined in the laboratory just how high a temperature was necessary and how long it had to be applied to kill the spores. This is the sort of study that our commercial fruit and vegetable canners use in determining the heat processing of canned foods.

After the time-temperature relation had been determined for **B. larvae** spores, it was possible to obtain from the data the time of heating needed to kill the spores at any temperature. A description of the determination of the thermal-death-time curve has been published elsewhere (5). Thus we had the type of information on which a process could be based.

We then developed a process, and our Engineering Section modified existing equipment so that it could be used for a continuous sterilization process for honey. Using a high-temperature short-time approach, we could kill the spores by heating the diluted honey[4] to 284° F. in two seconds, holding it at that temperature for 50 seconds, and then immediately cooling it to the boiling point of about 220°. In the small pilot-plant equipment, we could treat the diluted honey at a rate of 20 gallons per hour. Incidentally, this treatment had virtually no damaging effect on the honey (5). Honey treated in this manner, as well as similar untreated honey, was non-toxic when fed to bees in cages.

There is a catch to this process. In order to make it economically reasonable, the equipment has to be designed to treat several million pounds of honey per year. Of course, there isn't that amount of such honey at one place, and it isn't practical to ship honey for the purpose. It isn't the sort of thing a beekeeper could set up in the honey house.

Our final approach to the problem was to use the other end of our time-temperature curve. Instead of high temperature-short time, we used lower temperature-longer time. In order to eliminate costly equipment, we used the boiling point of honey when diluted to about 55% solids. From the thermal-death-time curve, for the temperature of 220° F., which is the boiling point at our altitude (about 500 feet above sea level) we found that the time required for sterilization, including a safety factor, is 90 minutes. This time would be increased with lower boiling points at higher elevations, as shown at the end of this article.

Some of our darker, off-grade honeys throw down a precipitate when they are boiled. This can be avoided if a small amount of phosphoric acid (an edible food acid) is added.

We prepared a quantity of honey carrying about 150 billion spores per liter, diluted it to 55% solids, acidified it with one fluid ounce of phosphoric acid (diluted 1 to 5 with water) per gallon of honey and boiled it for 90 minutes. The temperature increased from 218° to 227° F. during this time. Laboratory culture tests showed that there was no growth of **B. larvae** over a 30-day incubation period. Then it was submitted to the Bee Culture and Biological Control Laboratory at Laramie, Wyoming, along with some unheated infected control honey for feeding tests.

Colony Feeding Tests

THE FIRST colony feeding tests were started the latter part of May and early June 1952. Three healthy overwintered colonies of uniform strength were chosen. A careful inspection at that time showed no active disease or old disease scales. Two of the colonies were fed approximately two quarts each of the treated honey on two days a week apart, a total of four quarts. The third colony, used as a check, was fed two quarts of the spore-containing honey that had received no heat treatment.

The check colony had developed considerable AFB by the 19th day after being fed the untreated honey, and near the end of June was heavily diseased. Disease developed in both test colonies fed the treated honey. Six diseased cells appeared in one

[4] Honey was diluted to 55% solids; two parts by weight of honey to one part of water.

colony by the 19th day after being fed the treated honey. In the other colony disease developed much more slowly; only two AFB cells had appeared by the forty-fifth day after the first feeding.

Throughout July, disease spread very slowly in both colonies, only from two to five cells of AFB were seen at any one inspection. After the first of August until near the end of brood rearing in September, however, both colonies became relatively heavily infected. Since these colonies all were headed by queens of the same relatively susceptible stock, the eventual spread of disease in each colony, after the development of the slight amount of initial infection, was to be expected.

There are some explanations for the way disease developed in the two test colonies fed the treated honey. It is possible, although not probable, that a sufficient number of spores to produce the slight initial infections may have escaped the lethal conditions, either by adhering to the sides of the vessel above the liquid surface or in foam produced by the boiling, thereby remaining both viable and virulent. However, the sides of the vessel had been washed down several times.

Since the laboratory culture tests showed no growth of **B. larvae** even after 30-days' incubation, there is the more probable possibility that those test colonies may have picked up infection from some previous or outside source other than from the heated honey fed to them. The test colonies, although apparently healthy at the time they were fed the heated honey, may have carried over a small hidden focus of infection from **B. larvae** inoculations in connection with the American foulbrood resistance studies in previous years. An occasional case of disease the second season after inoculation has at times been observed in colonies with a similar history. Furthermore these colonies were located in an apiary in which some other AFB experimentally diseased colonies were present. The test colonies could have picked up infection either from drifting nurse bees or through robbing.

Since the test colonies were headed by AFB susceptible stock queens, a slight amount of infection in the colony or introduced from the outside might eventually produce active infection in a few larvae, from which the disease could spread through the colonies, as apparently appeared to be the case.

The treated honey tests made in 1952 were considered unreliable, since they were not sufficiently controlled to prevent the possibility of disease from sources other than the treated honey. Therefore, a second lot of AFB honey was prepared early in 1953 and sent to Laramie for further colony testing; the heating procedure and the **B. larvae** spore content were the same as used in 1952. In 1953, however, extra precautions were taken to prevent any contact of the bees with any source of infection other than what might be present in the treated honey fed to the colonies. The laboratory culture tests[5] were negative for growth of **B. larvae.**

SEVEN COLONIES were established for this purpose about the middle of May 1953 with two-pound packages of bees and sister queens, shipped from Louisiana. They were fed at the start only sugar syrup (one to one by volume of sugar and water), and each was given one pollen cake (pollen from a known non-infected source, mixed with soybean flour). After the colonies had developed to sufficient strength, six were moved to a completely isolated location some 15 miles from any disease-experiment apiaries or any other known source of AFB infection. The seventh colony was held at the original apiary location.

The six isolated test colonies were each fed two quarts of the 1953 heat-treated honey on July 1, and the seventh colony, used as a check, was fed two quarts of unheated honey on the same date. Some disease appeared in the check colony by July 10 and by July 21, three weeks after being fed the untreated honey, it was heavily diseased with American foulbrood. Inspections of the colonies fed the heat-treated honey were made about every 10 days throughout the 1953 season until the end of brood rearing. No disease was observed in any of these test colonies. All colonies con-

[5] Procedures for these tests are given in reference 5.

sumed the heated honey about as readily as sugar syrup, and no visible evidence of harmful effects to the brood or to the adult bees could be observed. All but one of the test colonies came through the winter of 1953 to 1954 in good condition. One colony died during the late winter from starvation. None of the remaining colonies showed any evidence of AFB when inspected on April 18 and again on June 3, 1954.

Therefore, results of one entire active season and observations well into the second season indicate that honey which may be contaminated with spores of **B. larvae** or honey from unknown sources, can be made safe for feeding to healthy colonies by the heat treatment described above. Colonies fed such treated honey should be kept under careful observation for more than one year. Such boiled honey should not be fed in winter because of the possible danger of its causing dysentery. Feeding such honey should be carried out only after bees can have unimpeded flights in the spring.

Process for Treating A.F.B. Honey

We can therefore outline the following process by which honey can be treated to permit feeding to bees without fear of spreading American foulbrood.

Diluted phosphoric acid (17%): To make, mix one part concentrated (85%) orthophosphoric acid with four parts of water.

Mix one 60-pound can of honey with 30 pounds of water (about 3½ gal.), add five fluid ounces of diluted phosphoric acid and heat to boiling. Note the temperature at which active boiling begins and continue boiling for the time given below.

215° F.	2¼ hours
220° F.	1½ hours
225° F.	1 hour

If much foam is formed, it should be skimmed off and disposed of, since spores could possibly survive the heating if they remain in a surface foam.

Acknowledgements

We are indebted to Eleanor J. Calesnick and Joseph Naghski for bacteriological work and to A. S. Michael, Floyd Moeller and J. D. Hitchcock for toxicity testing, and also to J. D. Hitchcock and I. L. Revell for assistance in colony manipulations and disease inspection.

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